Originals

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Morphological Comparison of *Asarum* sect. Asiasarum (Aristolochiaceae) in Japan with Special Reference to Multivariate Analyses of Flowers

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In order to clarify the morphological variation of plants belonging to *Asarum* sect. Asiasarum in Japan, we performed extensive field examinations for 55 populations covering both geographic range, taxa, and morphological races hitherto recognized. As previous studies were not enough in evaluation of quantitative characters, we performed multivariate analyses of various floral quantitative characters in addition to new qualitative floral characters, together with those used in the previous studies. As a result, eight morphologically distinct forms were recognized. They were first divided into two groups by color patterns in the inner surface of calyx tube; form D is dark purple, form L is dark purple proximally, ivory white, yellowish green or light purple in the middle part, dark purple or white at the throat. Form D is further divided into D1–D4 by the number of cells of trichomes in the adaxial surface of calyx and by the number of stamens and pistils. Form L is further divided into L1–L4 by the shape of calyx tube, the width of calyx tube throat, and the size and shape of calyx lobes. The eight forms were distributed almost allopatrically, and were concluded to be worthy to be recognized as distinct taxa.

Key words: *Asarum* sect. Asiasarum, color pattern, floral shape, multivariate analysis.

Asarum sect. Asiasarum Araki comprises six species and three varieties and occurs in northeastern Asia: Japan, China, Korea, and eastern Russia (Maekawa 1936b, Cheng and Yang 1983, Yamaki et al. 1996, Oh et al. 1997, Lee and Lee 2000). In Japan, three species and one variety: *A. sieboldii* Miq., *A. heterotropoides* F. Schmidt var. *heterotropoides*, var. *mandshuricum* (Maxim.) Kitag., and *A. dimidiatum* F. Maek. have been recorded so far (Maekawa 1956, Ohwi 1965, Satake and Momiyama 1982, Hatusima 1993). These taxa are classified mainly by leaf characters (outline, especially apex shape, variegated or not, and pubescence in the abaxial surface and petiole), calyx lobe characters (apex shape, recurved or erect), and the number of stamens and pistils.

In contrast, Nakamura (1986) applied unique attributes to evaluate the variation of sect. Asiasarum: floral, pollen, and chemical component characters rather than the foliar ones. In floral characters, she adopted calyx tube characters: inner and outer color pattern, constriction at the throat, the number

and distinctness of inner sculpture on inner surface, and the number of cells composing trichomes on inner surface. As a result of her extensive survey in Japan, she divided *A. sieboldii* into five floral forms: “Aso type”, “Tanigawa type”, “San’in type”, “Tohoku type”, and “Western Honshu type” (Table 1). Each form has an almost disjunct distribution from the others. In characters of pollen grains, Nakamura (1986) and Nakamura and Nagasawa (1987) reported the two forms of the supratecta corresponding to the floral forms. Type A pollen has smooth supratecta and is found in *A. heterotropoides* var. *heterotropoides* and the “Aso type”, “Tanigawa type”, “San’in type”, and “Tohoku type” of *A. sieboldii*. Type B pollen has striated supratecta and is found in “Western Honshu type” *A. sieboldii* and *A. dimidiatum*. In chemical component characters, geographic variations of phenylpropanoids and monoterpenoids were examined for *A. heterotropoides* var. *heterotropoides* (Nakamura et al. 1979), *A. dimidiatum* (Nakamura et al. 1982), and *A. sieboldii* (Nagasawa 1961, Nakamura et al. 1987). In *A. sieboldii*, the proportion of two phenylpropanoids (methyleugenol : safrole) had geographically clinal variations, and three distinct monoterpenoid forms were recognized (Nakamura et al. 1987). Floral, pollen, and chemical component characters almost corresponded to each other (Nakamura 1986). In conclusion, she recognized seven forms in Japan.

However, the study is insufficient in comparison of quantitative characters, especially the size and shape of each part. For example, although “Tanigawa type” and “Tohoku type” *A. sieboldii* were discriminated by constriction at throat of calyx tube and distinctness of longitudinal sculpture in inner surface of calyx tube, the delimitation of these characters between them were not presented clearly. As the result, it is difficult to clarify plants into each form without geographic information.

The aim of this study is to clarify the morphological variation of *Asarum* sect. Asiasarum in Japan. For the purpose, we performed extensive field examinations both in geographic range and number of individuals.

To recognize distinct forms in quantitative characters, we newly applied one of the multivariate analyses, canonical variates analysis (CVA). In cases where each of the examined populations is highly expected to be composed of individuals in a single group, CVA with each population as an *a priori* group makes it possible to know the boundaries between distinct groups without any *a priori* grouping by precedent systems (Wiley 1991).

Materials and Methods

Samples examined

Field collections were performed on 55 populations (Fig. 1, Table 2) in flowering season from 1997 to 2001 to cover almost all areas where sect. Asiasarum plants were reported in Japan.

In field collections, ephemeral characters such as color patterns of calyx tubes were observed and color photographs were taken before fixing or pressing. Flowers were fixed with FAA (formaldehyde: acetic acid: 50 % ethanol = 1 : 1 : 18) and were then used for observation and measurement. A number of individuals were cultivated. Voucher specimens are deposited in the Herbarium of Tohoku University (TUS).

Measurements and observations of floral attributes

Fourteen quantitative floral attributes were measured for 731 individuals from 55 populations. More than half the characters are included in previous studies (calyx tube width and length, calyx tube throat width, calyx lobe width and length, ovule position, pistil protuberance width: Nakamura 1986, Yamaki et al. 1996: Fig. 2), and a part of data for *A. heterotropoides* var. *hetero-*

Table 1. Comparison between the system of *Asarum* sect. Asiasarum in Japan, and diagnostic characters and distribution of the taxa recognized by Nakamura (modificated from Nakamura 1986)

	A. heterotropoides	*Asiasarum sieboldii*					*A. dimidiatum**
	A. heterotropoides	Aso type***	Tanigawa type	San'in type	Tohoku type	Western Honshu type	*A. dimidiatum***
Flower characters							
Constriction at the throat of calyx tube	+++	+++	+++	+++	++	+	+
Color of calyx-tube inside	yellowish green-pale purple	yellowish green	pale purple	dark purple	yellowish green	dark purple	dark purple
Number of cells of floral trichomes	5	3	5	7–9	3	1	3
Pollen character							
Presence of the striate on the surface of verruca	smooth	smooth	smooth	smooth	smooth	striated	striated
Essential oil							
Main components of the monoterpenoids	eucarvone	borneol	eucarvone	1,8-cineol α-terpinenol terpinenol-4	eucarvone	1,8-cineol α-terpinenol terpinenol-4	1,8-cineol α-terpinenol terpinenol-4
Distribution	Honshu (Tohoku district), Hokkaido, Sakhalin and the S. Kuriles	Kyushu (Kumamoto Pref.)	Honshu (Tochigi, Fukushima, Gunma and Nagano Prefs.)	Honshu (Shimane and Hiroshima Pref.)	Honshu (north of central Honshu)	Honshu (southwest of central Honshu) and Tsushima Island	Kyushu, Shikoku and Kii Peninsula

*Maekawa (1936b), Satake and Momiyama (1982).
**Nakamura (1986).
***"Aso type" *A. sieboldii* populations were recognized *A. heterotropoides* var. *mandshuricum* by Hatusima (1993).

Table 2. Locality, number of individuals of which floral features were measured, and voucher specimen of each population examined in this study

No.	Locarity	n	Voucher specimen	Precedent studies*
HOKKAIDO				
1	Soya, Hamatonbetsu-cho	18	HY 6301	1, 3: HET
2	Kamikawa, Nayoro-shi	19	HY 6300	1, 3: HET
3	Abashiri, Kitami-shi	11	HY 6302	
4	Abashiri, Kiyosato-cho	19	HY 6303	1, 3: HET
5	Kamikawa, Asahikawa-shi	13	HY 6299	1, 3, 4: HET
6	Sorachi, Kuriyama-cho1	2	HY 7119	
7	Sorachi, Kuriyama-cho2	9	HY 6304	
9	Tokachi, Nakasatsunai-mura	6	HY 6309	
10	Tokachi, Makubetsu-cho	2	HY 7133	
8	Hidaka, Hidaka-cho	5	HY 6308	
11	Hidaka, Biratori-cho	10	HY 7122	1
12	Hidaka, Niikappu-cho	18	HY 6280	
13	Hidaka, Shin-hidaka-cho	4	HY 6291, 7113	
14	Hidaka, Samani-cho 1	8	HY 4062	
15	Hidaka, Samani-cho 2	10	HY 4169, 7137	1
16	Hidaka, Samani-cho 3	8	HY 4155	
18	Hidaka, Samani-cho 4	13	HY 4142, 7105	
17	Hidaka, Erimo-cho	20	HY 7135	
19	Shiribeshi, Otaru-shi	16	HY 6297	
20	Oshima, Hakodate-shi	18	HY 6293, 7101	
HONSHU				
21	Aomori, Mutsu-shi	10	HY 7023	
22	Aomori, Hirosaki-shi	19	HY 6160	1
23	Aomori, Noheji-machi	4	HY 6150	1, 3: HET
24	Akita, Akita-shi, Mt. Taihei, alt. 300 m	21	HY 6175	5
25	Akita, Akita-shi, Mt. Taihei, alt. 900 m	21	HY 6170	1, 3, 4: HET
26	Iwate, Ofunato-shi	21	HY 6065	
27	Yamagata, Tsuruoka-shi	10	HY 6100	5
28	Yamagata, Nishikawa-machi	11	HY 6078	
29	Miyagi, Kurihara-shi, Mt. Kurikoma	13	HY 6120	3: HET
30	Fukushima, Fukushima-shi	22	HY 6060	3, 4, 5: TOH
31	Tochigi, Tamura-shi	17	HY 7017	
32	Gunma, Katashina-mura	20	HY 6262	
33	Niigata, Minamiuonuma-shi	17	HY 7014	
34	Nagano, Myoko-shi, Mt. Madarao	11	HY 6234	3, 5: TAN
35	Niigata, Itoigawa-shi	21	HY 7008	
36	Nagano, Sakae-mura	9	HY 6220	3, 5: TAN
37	Nagano, Kishimataira-mura	3	HY 9004	
38	Nagano, Otari-mura	10	HY 6045	3, 4, 5: TOH
39	Nagano, Hakuba-mura	19	HY 7010	3, 5: WES
40	Nagano, Nakano-shi	11	HY 7013	
41	Nagano, Nagano-shi	10	HY 7012	
42	Nagano, Okuwa-mura	16	HY 7005	
43	Nagano, Ina-shi	15	HY 6050	3, 4, 5: WES
44	Nagano, Iida-shi	10	HY 6051	3, 4, 5: WES
45	Toyama, Tonami-shi	20	HY 7007	
46	Ishikawa, Hakusan-shi	18	HY 7006	3, 4, 5: WES
47	Gifu, Gujo-shi	20	HY 6035	
48	Tottori, Tottori-shi	12	HY 6016	3, 4, 5: WES
49	Shimane, Okuizumo-cho, Uchitani	11	HY 7002	3, 4, 5: SAN
50	Hiroshima, Miyoshi-shi	15	HY 7004	
SHIKOKU				
51	Ehime, Matsuyama-shi, Mt. Takanawa	15	HY 8004	2: DIM
KYUSHU				
52	Fukuoka, Soeda-machi, Mt. Hiko	9	HY 6190	2: DIM
53	Ooita, Beppu-shi, Mt. Kuraki	20	HY 8007	
54	Kumamoto, Aso-shi 1	9	HY 6200	3, 4, 5: ASO
55	Kumamoto, Aso-shi 2	12	HY 6215	

*Populations used in precedent studies were marked with used names as follows: 1: Nakamura et al. (1979), 2: Nakamura et al. (1982), 3: Nakamura (1986), 4: Nakamura and Nagasawa (1987), 5: Nakamura et al. (1987). Those used in Nakamura (1986) were identified followed by the system of Nakamura (1986); HET: *A. heterotropoides* var. *heterotropoides*, TOH: “Tohoku type” *A. sieboldii*, TAN: “Tanigawa type” *A. sieboldii*, WES: “Western Honshu type” *A. sieboldii* , SAN: “San’in type” *A. sieboldii*, ASO: “Aso type” *A. sieboldii*, DIM: *A. dimidiatum*. HY is an abbreviation of “H. Yamaji”.

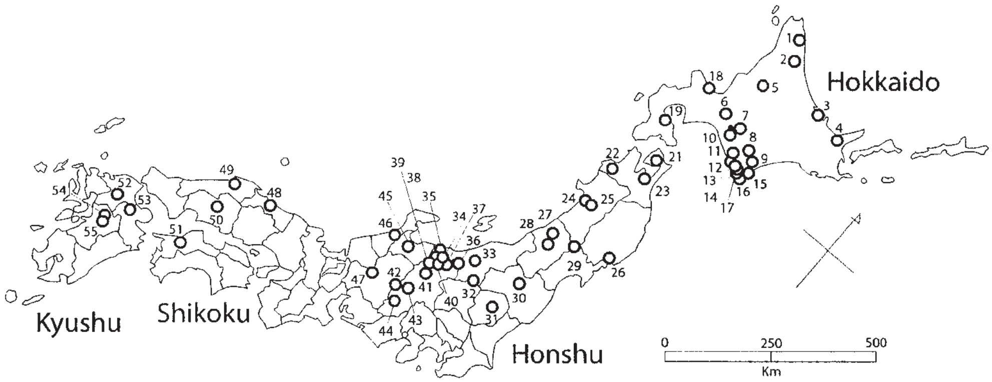

Fig. 1. Populations examined of *Asarum* sect. Asiasarum in this study.

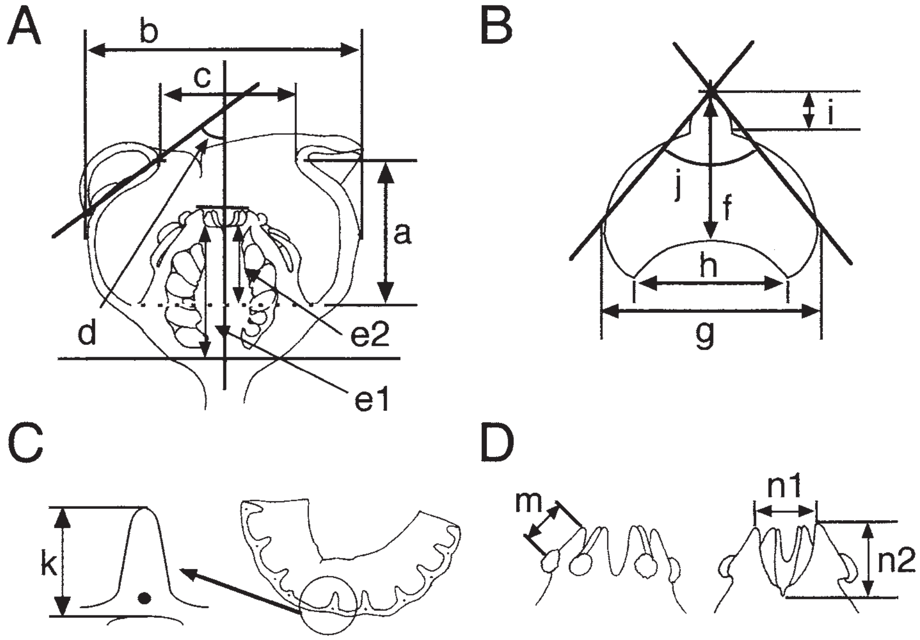

Fig. 2. Measurement parts of flowers in *Asarum* sect. Asiasarum. A: Vertical section of flower, a: calyx tube length (CTL), b: calyx tube width (CTW), c: calyx throat width (CTTW), d: degree of calyx lobe (CLD), e: ovule position (e2/e1: OP). B: Flattened calyx lobe, f: calyx lobe length (CLL), g: calyx lobe width (CLW), h: calyx lobe foot width (CLFW), i: tip length (CLTL), j: tip degree (CLTD). C: Ridges in inner surface of calyx tube, k: height (RH), l: number (RN). D: Pistil, m: pistil protuberance length (PPL), n: pistil protuberance inclination (n1/n2: PPI).

tropoides was quoted from Yamaji et al. (2006).

The procedure of measurement was same as Yamaji et al. (2006). For vertical sections of flowers (Fig. 2A), flowers were cut longitudinally, and images of the sections were

digitized using a flat bed scanner (EPSON GT-7000S). Coordinates of 11 landmarks were acquired from the digital images, and the value of each character was calculated from the coordinates. The same procedure was used to characterize the calyx lobes (Fig. 2B). Each calyx lobe was placed between glass slides, and its image was directly digitized by the flat bed scanner. The lengths of pistils and the height of ridges on the inner surface of the calyx tube were measured with a fine plastic measure to 0.1 mm accuracy (Figs. 2C, 2D). Trichomes on inner surface of calyx tube and lobes were observed preliminary in a limited number of samples using a scanning electron microscope (SEM: Hitachi S-4100), and were subsequently confirmed in the other samples using light microscopes. For observation by the SEM, fixed flowers were dehydrated with ethanol/ isoamyl acetate series, and were dried in a critical-point dryer (Hitachi HCP-1). Then, they were put on metal holders with adhesive tapes, and were coated with Pt-Pd (Ion Sputtering Machine: Hitachi E-1030). These prepared samples were observed by the SEM at 2–10kv. For observation by light microscope, fixed flowers were sliced by a freeze-microtome (Yamato Koki, Komatsu Electronic Inc. MA-101) were put on slide glasses with 50 % glycerol, and were sealed by cover glasses with a mounting reagent (Eukitt: O. Kindler).

Data analysis

We aimed to establish a hypothetical grouping for the measured populations using both qualitative and quantitative characters. Canonical variates analysis (CVA) was performed with each population as an *a priori* group. The presumption of the CVA, uniformity of each population, was decided by field observations. The CVA was performed with the computer program package STATISTICA 4.1J for Macintosh (Statsoft Inc.) and SPSS 9.0 for Windows (SPSS Japan Inc.). The characters were standardized manually by subtracting the total mean from an individual raw score and then dividing the difference by the total standard deviation, so that each variable had a mean of unity. To recognize discrete groups more efficiently, the measured populations were also divided into several categories by diagnostic qualitative characters beforehand, and a new CVA was performed for each category.

After a hypothetical grouping was established, the grouping was compared with the other characters, to find available diagnositic characters.

Results

Characters other than floral quantitative characters

1. Habit In basic structure of plants belonging to *Asarum* sect. Asiasarum in Japan, there is no difference in habit among the observed materials in this study and the descriptions by Maekawa (1936b) and Kelly (1997). They are low-growing, rhizomatous herbs with distinctive, modular growth. Flowering modules usually consist of almost fixed number of foliar organs: normally three scale leaves, two foliage leaves, and a solitary, terminal flower. Sterile modules consist of 2–3 scale leaves and one foliage leaf. Aberrant exceptions were recognized in a few individuals in populations Nos. 47 and 54; they had three leaves in each module. Rhizome is hairless, about 5 mm in diameter, with leaf and flower trace and sometimes branching. Roots are usually about 1 mm in diameter, fleshy, slender, distributed evenly along rhizomes. Cataphylls are caducous, glabrous, ciliate in the margin, triangular ovate. Flower is solitary terminal, actinomorphic. Peduncle is glabrous. Sepals are three, connate, forming calyx tube; calyx tube is glabrous in outer surface and striated longitudinally, internally ridged.

2. Foliage leaves Foliage leaves of this group in Japan were almost similar: deciduous, membranous, alternate, and petiolate.

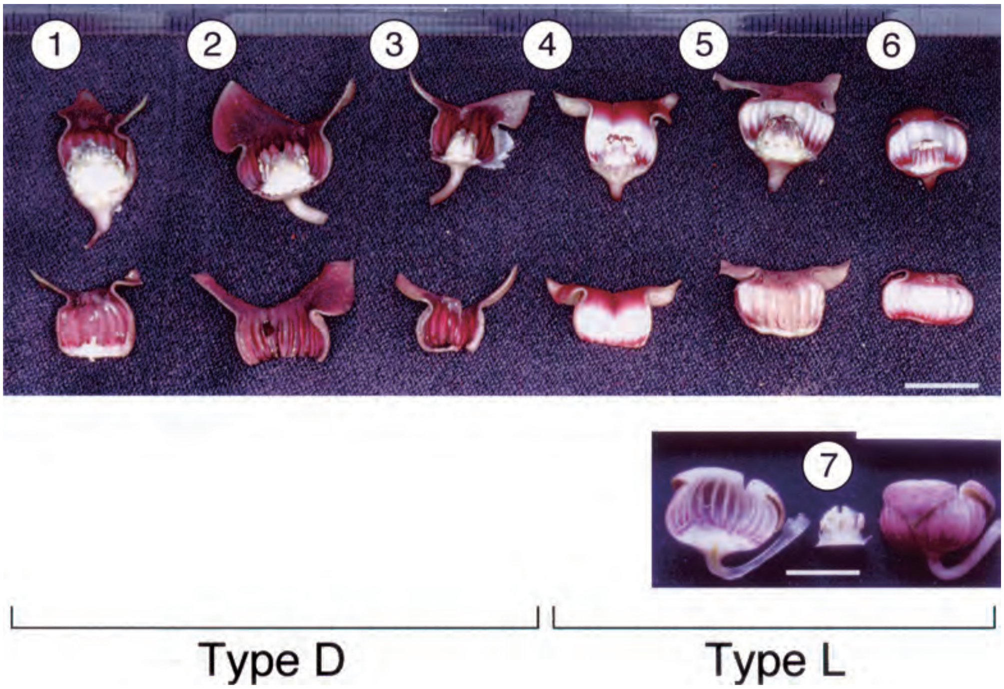

Fig. 3. Variation of color pattern in inner surface of calyx tube in *Asarum* sect. Asiasarum. Bar = 1cm. Form D. 1: population No. 47, 2: No. 43, 3: No. 51. form L. 4: No. 26, 5: No. 33, 6: No. 19, 7: No. 53.

Laminae are simple, palmate veined, ciliate along the margin. Proximal and distal laminae have different trend in outline: proximal lamina ovato-cordate–broadly cordate, on the other hand, distal lamina pentangural ovate cordate. They were highly variable in size even in a single population. In foliage leaves, Maekawa (1936b) recognized that obtuse or acute (not acuminate) apex was characteristic of *A. heterotropoides* var. *heterotropoides*. However, we found many intermediate forms in plants belonging this taxon from southeastern Hokkaido (Yamaji et al. 2006) and northern Honshu (Nakamura 1986).

All plants and specimens examined in this study have laminae sparsely pilose on the adaxial surface and on the veins of abaxial surface, and petioles are pilose in only adaxial ridges, though pubescens of leaf laminae and petioles are used to discriminate plants belonging to this group in Korea and China (Maekawa 1936b, Cheng and Yang 1983, Yamaki et al. 1996). Consequently, it was difficult for the plants to be divided into several distinct states with such leaf characters alone, and we compared the variation of them after a hypothetical grouping was established with the other characters.

3. Position of hibernaculum There are two different forms in the position of the hibernaculum. In almost all cultivated individuals from the populations examined, the axillary bud of the most distal foliage leaf dominantly developed into hibernaculum, and those of the proximal leaf and cataphylls sometimes develop secondarily. On the other hand, in the individuals from two populations in central Kyushu (Nos. 53, 54), axillary buds of cataphylls dominantly

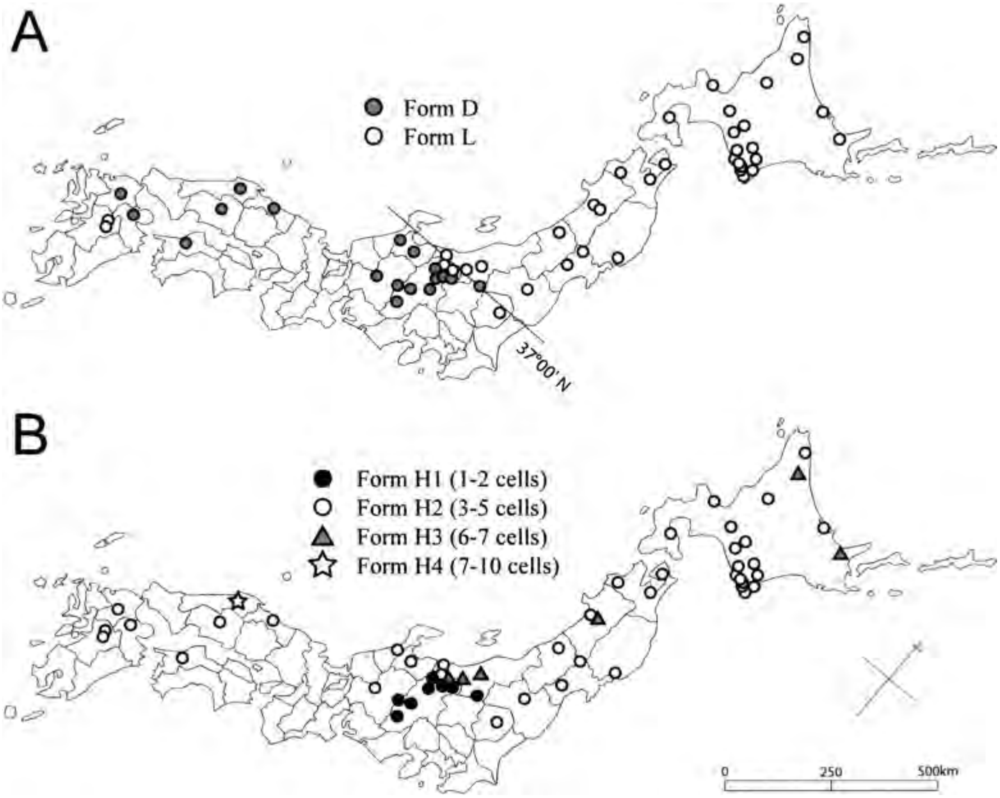

Fig. 4. Geographic variation of *Asarum* sect. Asiasarum in Japan. A: Color pattern of flower. B: Number of cells of each multicelluar hair in adaxial surface of calyx tube and lobes.

developed instead and those of foliage leaves did not develop. This difference probably affects the shape of rhizomes; the former tends to grow straight, the latter tends to grow zigzag.

4. Floral color pattern In color patterns of internal surface of calyx tube, two distinct forms, we call forms D and L hereafter, were observed (Fig. 3). The pattern of form D was as follows: ivory white or greenish yellow at the base, dark purple proximally, in the middle part, and at the throat. The pattern of form L was as follows: ivory white or greenish yellow at the base, dark purple proximally, ivory white, yellowish green, light purple, or their intermediate in the middle part, dark purple or rarely ivory white at the throat. Ivory white throat were recognized in all the individuals of Nos. 53 and 54, and rarely in Hokkaido populations. Therefore, the two forms were clearly distinct in the middle part: dark purple or not. These color patterns were stable within a single population.

The distribution of the two forms were almost allopatric (Fig. 4A). Form D was distributed in central and western Honshu, Shikoku, and Kyushu. Form L was distributed in Hokkaido, northern and eastern Honshu, and disjunctively in central Kyushu. The border of the two forms in Honshu lay near the 37°00´N parallel.

In contrast, the color of outer surface of the calyx tube varied even in a single population. Nevertheless, four distribution pattern were recognized: (1) bright dark purple,

mainly in Hokkaido, (2) bright ivory pink, light purple, or their intermediates, in central Kyushu and northern Honshu, (3) yellowish brown with purple dots, olive green with purple dots, purple with yellowish green dots, or their intermediates, in central and northern Honshu, (4) grayish olive green with purple dots, in central and western Honshu, Shikoku, and Kyushu. The color pattern combinations of adaxial and abaxial surface of calyx lobes also varied even in a single population. The abaxial surface was generally the same as or brighter colored than that of outer surface of calyx tube. However, the color of the adaxial surface seemed to have no relation to that of the abaxial surface and varied from ivory pink, dark purple to light green, or intermediate colors.

Individuals in the greater part of the populations had pistil protuberances colored dark purple to greater or less degrees. On the other hand, populations in central Kyushu (Nos. 53, 54), and almost all individuals in a population in western Honshu (No. 48) were unique in having yellowish green protuberances.

5. Trichomes of flowers The number of cells in each trichome on the inner surface of the calyx tube and adaxial surface of calyx lobes varied remarkably among the populations but was stable within a single population. In the precedent studies, this character was simply categorized into four forms; each trichome was composed of (1) single, (2) three, (3) five, or (4) seven to ten cells (Nakamura 1979, 1986, Table 1). However, the result in this study was rather variable; the number of cells of trichomes sometimes differed about ± one even within an individual. Consequently we divided trichome conditions into four forms (Fig. 5). The number of cells in each form was as follows: one or two (form H1), three to five (rarely more than five, form H2), six or seven (rarely eight, form H3), and seven to ten (sometimes more than ten, form H4). The distribution of each form was less allopatric than that of the color pattern in the inner surface of calyx tube (Fig. 4B); form H1 was observed in eight populations from central Honshu, form H2 was in 40 populations from almost all areas examined, form H3 was in six populations in narrow area of central Honshu, northeastern Honshu, and Hokkaido, and form H4 was in only one population in western Honshu (No. 48).

6. The number of stamens and pistils As in the other sections of *Asarum*, the regular number of stamens and pistils were 12 and 6, respectively, and the greater part of populations examined showed these standard numbers: standard S (stamens) & P (pistils). On the other hand, in three populations (Nos. 50, 51, and 52), they are reduced to half: 6 and 3, respectively: reduced S & P. Reduction of stamens and pistils is regarded as one of the diagnostic characters to discriminate *A. dimidiatum* from the other species (Maekawa 1936a, 1936b, Ohwi 1965, Satake and Momiyama 1982).

7. Conclusion of the characters other than floral quantitative traits Among the characters mentioned above, the floral color pattern in inner surface of calyx tube was most discrete and stable, and the two forms were distributed in different areas. Therefore we classified populations into forms D and L firstly, and further subdivided them by the other characters, and compared with CVA results.

Form D populations involved a wider range of variation than form L populations in the qualitative characters examined in this study. In the condition of trichomes of flowers, form D populations were divided into three forms, form H1 (eight populations), form H2 (eight populations), and form H4 (one population). In the number of stamens and pistils, fourteen populations had standard S & P, and three populations had reduced S & P. In the combination of these two

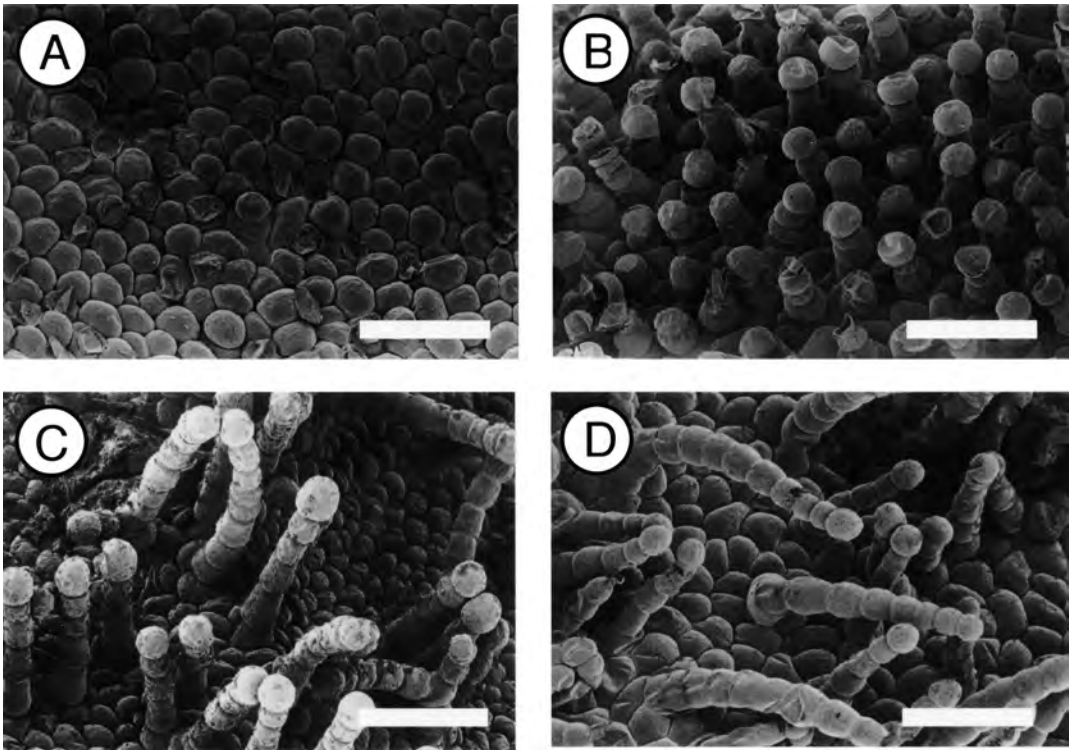

Fig. 5. SEM phtographs of hairs on inner surface of calyx lobe in *Asarum* sect. Asiasarum. A: Form H1 (population No. 39). B: Form H2 (No. 24). C: Form H3 (No. 38). D: Form H4 (No. 48). Bar = 100 μm.

characters, four forms were recognized in form D populations: form D1 (form H1, standard S & P, eight populations), form D2 (form H2, standard S & P, five populations), form D3 (form H4, standard S & P, one population), and form D4 (form H2, reduced S & P, three populations). The four D forms were almost allopatrically distributed (Fig. 6). Form D1 was distributed in central Honshu, form D2 was in western Honshu, form D3 was restricted to western Honshu, and form D4 was in Shikoku and Kyushu.

Form L populations were less variable in the characters examined above. Though they are divided into two forms, form H2 (32 populations) and H3 (six populations), in the condition of trichomes, the distribution and relationship to the other characters were less clear. Moreover, the other characters are difficult to classify the form L populations into more than one distinct group.

Canonical variates analyses (CVA) for floral quantitative characters

Through the field observations, we found that each population was most likely composed of only a single morphological form because the shape, the proportions of variables, and most of the qualitative characters of flowers mentioned above were stable in each population (Yamaji unpubl. data). This condition enabled us to designate each population as an a priori group for CVA (Wiley 1991).

1. Total populations Fourteen significant ($p < 0.00001$) CVs were calculated. The variances accounted for the axes I–III were 45, 22, and 8 %, respectively. The eigen-

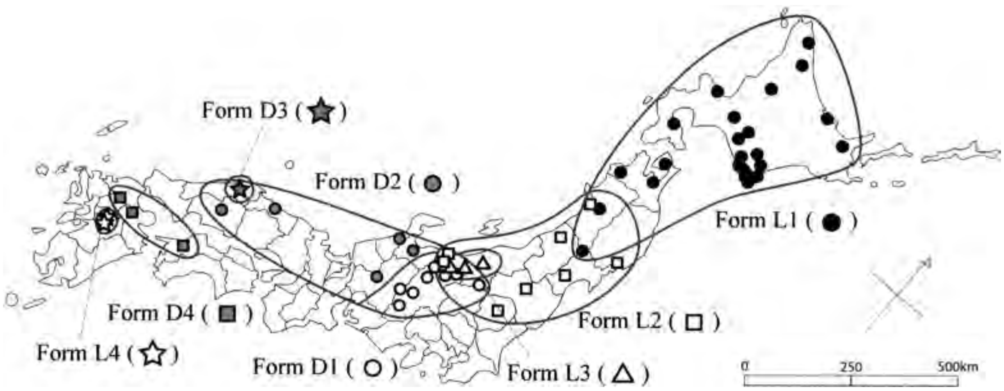

Fig. 6. Recognized forms of *Asarum* sect. Asiasarum in Japan in combination with color form, hair form and the CVA results.

Table 3. The standardized eigenvector and eigenvalues for the first three caronical variables in *Asarum* plants examined in this study

Variables	**A. Total populations** Axes I	II	III	**B. Color form D populations** Axes I	II	III	**C. Color form L populations** Axes I	II	III
Ovule position (OP)	0.300	0.277	0.277	–0.434	0.023	–0.273	–0.018	–0.346	0.004
Calyx tube width (CTW)	–0.824	0.154	0.664	–0.212	–0.677	–0.302	0.747	–0.160	0.086
Calyx tube length (CTL)	–0.141	–0.781	0.101	0.474	–0.713	0.145	–0.382	0.352	0.556
Calyx tube throat width (CTTW)	0.642	0.160	–0.283	–0.573	0.356	0.133	–0.444	–0.315	–0.083
Degree of calyx lobe (CLD)	0.277	–0.307	–0.400	0.007	0.139	0.378	–0.515	0.251	–0.188
Calyx lobe length (CLL)	0.181	0.073	–0.479	–0.835	–0.416	0.724	–0.177	0.167	–0.314
Calyx lobe width (CLW)	0.472	–0.449	–0.025	0.510	0.060	–0.471	–0.342	0.447	–0.343
Calyx lobe foot width (CLFW)	0.159	0.106	0.641	–0.151	0.028	–0.049	–0.152	–0.811	0.503
Tip degree (CLTD)	–0.075	–0.016	0.190	–0.079	–0.048	–0.127	0.113	–0.050	0.122
Tip length (CLTL)	0.032	–0.002	0.048	–0.017	0.016	–0.213	–0.012	–0.008	–0.104
Pistil protuberance length (PPL)	0.160	0.465	–0.254	–0.195	0.644	–0.125	0.013	–0.278	–0.431
Pistil protuberance inclination (PPI)	0.151	–0.074	0.328	0.005	–0.307	–0.476	–0.082	0.030	0.433
Calyx tube ridges height (RH)	–0.376	0.729	–0.141	0.855	0.765	–0.425	0.647	–0.023	–0.345
Calyx tube ridges number (RN)	0.310	–0.075	0.056	–0.037	0.216	–0.517	–0.374	–0.147	–0.335
Eigenvalues	16.256	7.778	3.056	11.185	8.085	5.313	12.703	5.285	2.652
Percentage of total variance	44.9 %	21.5 %	8.4 %	34.4 %	24.9 %	16.4 %	47.6 %	19.8 %	9.9 %

vectors of each variable to CV I, CV II, and CV III are shown in Table 3A. The two-dimensional scatter diagrams on CV I & II and CV I & III are shown in Figs. 7A and 7B, respectively. The ranges of populations overlapped with each other, and any clusters were hardly recognized in the diagrams. This unclear result may indicate the limit of CVA for simultaneous recognition of many groups. Therefore, as elucidated above, combined analyses with the other characters examined above possibly act effectively to discriminate morphologically distinct groups.

The most clear-cut quantitative character, color pattern in inner surface of calyx tube (forms D and L) divided both the individuals and populations into different ranges effec-

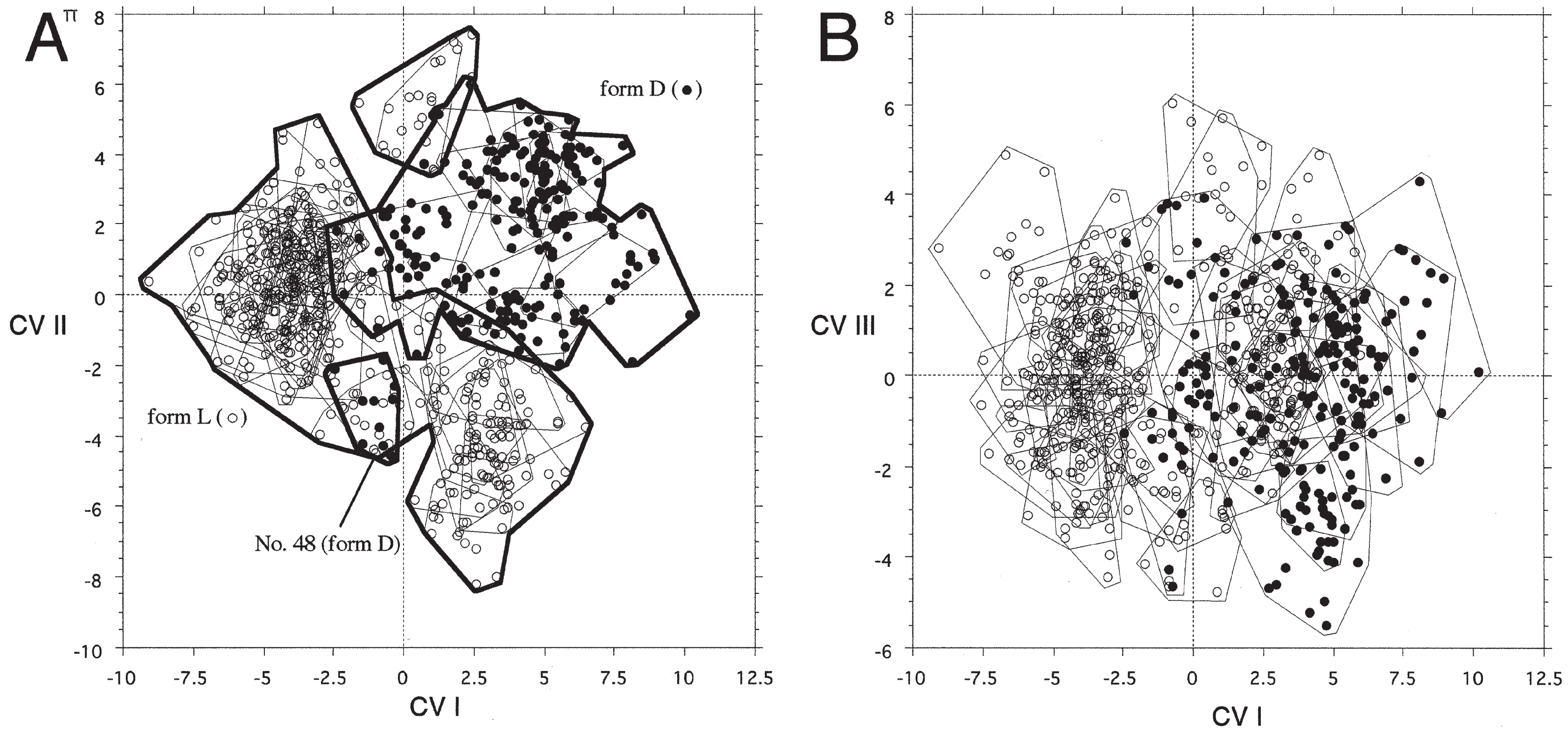

Fig. 7. Two-dimensional scatter of the samples of *Asarum* sect. Asiasarum on canonical variate axes. A: All populations, CVI & CV II. B: All populations, CV I & CV III. Individuals in A and B are distinguished by the color forms in inner surface of calyx tube (form D & L).

tively in the CV I & CV II scatter diagram (Fig. 7A). Form D individuals mainly occupied in positive field in both of the two CVs except one population (No. 48, as mentioned above, the unique population with form H4 hairs), and form L ones occupied in the other field. This result indicates that individuals different in floral color pattern are also different in the shape and/or size.

2. Color form D populations Ten significant ($p < 0.00001$) CVs were calculated. The variances accounted for the axes I–III were 34, 26, and 16 %, respectively. The eigenvectors of each variable to CV I, CV II, and CV III are shown in Table 3B.

In the two-dimensional scatter diagram on CV I and CV II (Fig. 7C), any clusters were hardly recognized. This result possibly indicated that there is no clear border to divide form D populations into more than one group in floral quantitative variation alone. In the diagram, most form D1 populations were in negative field to around neutral position in the both axes, on the other hand, form D2 populations were around neutral position to positive field in both axes. Form D3 population was in the positive field in CV I and negative field in CV II. Form D4 populations were around neutral position of CV I and around neutral position to positive field of CV II, and overlapped with both forms D1 and D2. Among the coefficients of CV I, highly negative factors were seen in characters CLL, CTTW, and OP, on the other hand, highly positive factors were in RH, CTW, and CTL. As well, among the coefficients of CV II, highly negative factors were in CTL, CLW, CLL, and PPI, and highly positive factors were in RH, PPL, and CTTW.

In the two-dimensional scatter diagram on CV II and CV III (Fig. 7D), which was effective to make clusters between the populations, it should be noted that form D3 was separated in the positive field in CV II and in the negative field in CV III. Among the coefficients of CV III, highly negative factors were seen in characters CTW, CLW, PPI, RH, and RN, and highly positive factors were in CLD and CLL. The difference of distribution range of each form in the scatter diagrams indicates that the four forms were continuous but different in the floral quantitative characters, which supports the difference in morphological variation of among form D populations.

3. Color form L populations Eleven significant ($p < 0.00001$) CVs were calculated. The variances accounted for the axes I–III were 48, 20, and 10 %, respectively. The eigenvectors of each variable to CV I, CV II, and CV III are shown in Table 3C.

The two-dimensional scatter diagram on CV I & CV II is shown in Fig. 7E. In the scatter diagram, four clusters were recognized. The result of comparatively clear clusters was different from that in form D populations; the overlap area between the populations was remarkably larger than that of form D.

Three clusters were recognized along the axis CV I. The first cluster (form L1) in the positive field was composed of 24 populations. The second cluster (form L2) in the negative field was composed of six populations. The third cluster (form L3) was in the intermediate position between the first and the second clusters, and was composed of three populations. Though the three clusters overlapped in their margins, a few aberrant exceptional individuals seemed to cause these overlaps. In addition, their median points were obviously in different positions. Therefore, we decided that they are enough to be hypothetical distinct forms for the first step of the following analyses. Among the coefficients of CV I, highly negative factors were seen in characters CLD, CTTW, CTL, RN, and CLW, and highly positive factors were in CTW and RH.

Two populations in central Kyushu (Nos. 53, 54) were separated in the negative field of CV II from the other populations, and

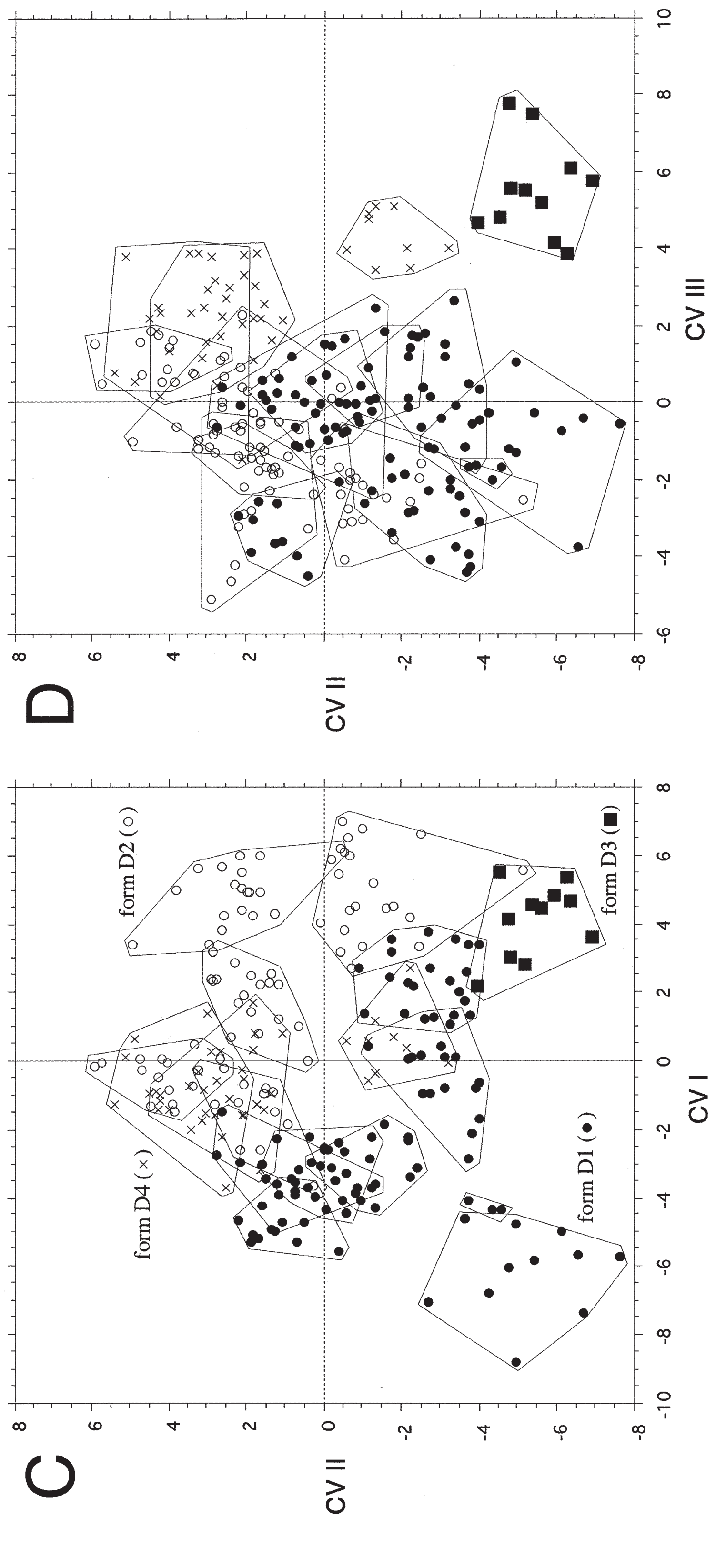

Fig. 7. Continued. C: Form D populations, CV I & CV II. D: Form D populations, CV I & CV III. Individuals in C and D are distinguished by the floral trichome conditions and the number of stamens and pistils.

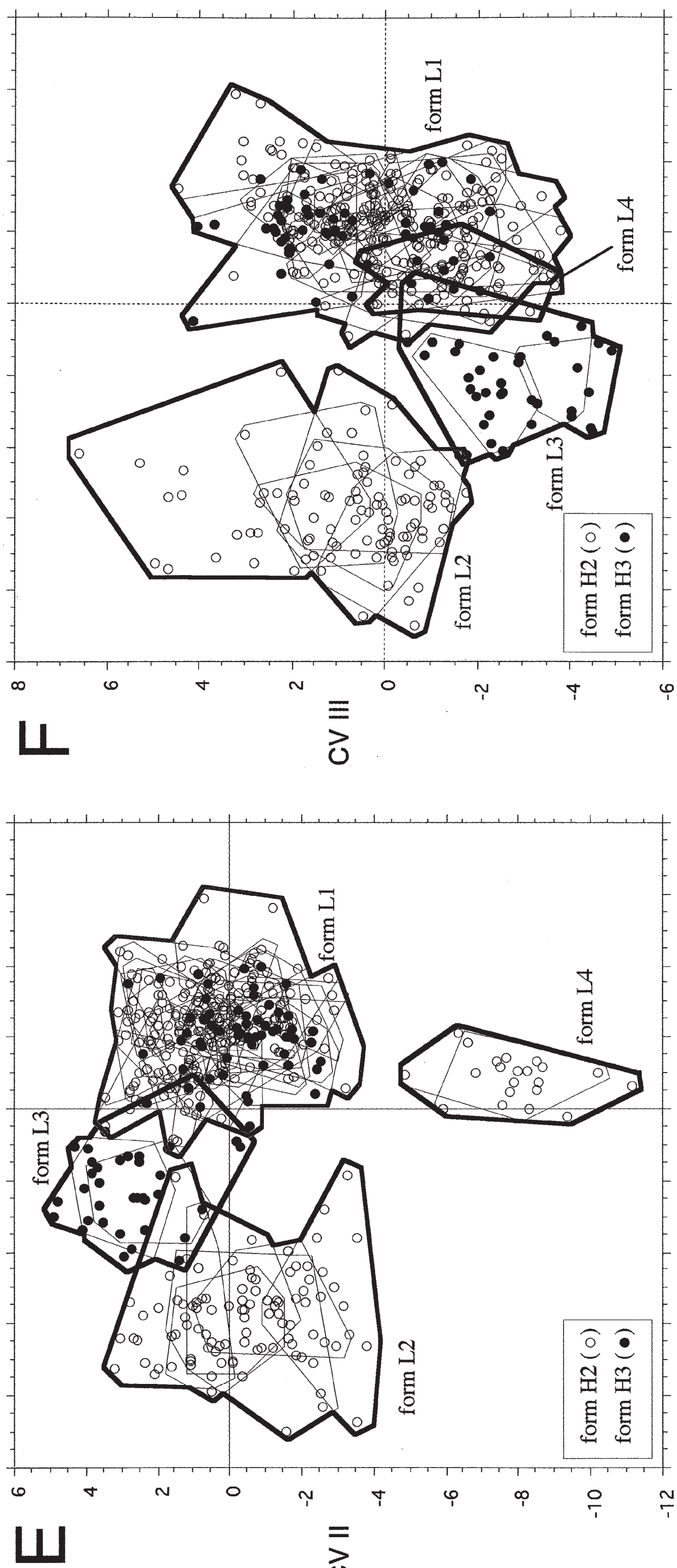

Fig. 7. Continued. E: Form L populations, CV I & CV II. F: Form L populations, CV II & CV III. Individuals in E and F are distinguished by the floral trichome conditions.

range of these two populations strongly overlapped each other; we call them form L4. Among the coefficients of CV II, highly negative factors were seen in characters CLFW, CTTW, and PPL, and highly positive factors were in CLW, CTL, and CLD.

The two-dimensional scatter diagram on CV I and CV III, which was effective to make clusters between the populations, is shown in Fig. 7F. In this diagram, form L4 was completely immerged into the range of form L1, while form L2 and L3 occupied different range in CV III. Among the coefficients of CV III, highly negative factors were seen in characters PPL, RH, CLW, RN, and CLL, highly positive factors were in CTL, CLFW, and PPI.

These four forms could be recognized more conspicuously by the combination of other qualitative characters mentioned above. In the position of hibernaculum, those of form L4 were dominantly in axillary buds of cataphylls, on the other hand, those of the other forms were dominantly in axillary buds of distalmost foliage leaf. In trichomes of flowers, two forms were observed in L forms (Figs. 7E, 7F). All populations belonging to form L3 and six of 24 populations belonging to form L1 had form H3 trichomes. The other populations had form H2 trichomes. Although form L3 was overlapped with form L1 and L2 in the margin in the scattered diagram of CV I and CV II, it could be distinguished from at least form L2 in this character.

The four forms had slightly differing shape in foliage leaves especially at their apex. Form L4 stably had obtuse or acute leaves at apex, and forms L2 and L3 stably had leaves at the acuminate apex. On the other hand, form L1 had various leaves in apical shape; individuals in northern Honshu and southeastern Hokkaido tended to have acuminate leaves at apex, but the other individuals tended to have leaves obtuse or acute at the apex. The stability of leaf forms also varied among populations.

In the color of calyx lobes and outer surface of the calyx tube, the four forms had different trends. Calyx tubes and lobes of form L4 individuals were ivory pink, light purple, or their intermediate colors without any mixture of desaturated colors, e. g., olive green. Those of form L1 were roughly divided into three forms recognized in different areas: (1) fine ivory pink, light purple, or their intermediate colors found in northern Honshu, (2) desaturated olive green with purple dots, purple with yellowish green dots, or their intermediate colors, calyx lobes sometimes light green found in southeastern Hokkaido, (3) uniformly fine dark purple seen in almost all area of Hokkaido. However, they were continuous with intermediate individuals. Those of forms L2 and L3 were variable even in one population, yellowish brown with purple dots, olive green with purple dots, purple with yellowish green dots, or their intermediate colors. Calyx lobes of form L3 were sometimes pale green.

Univariate analyses

The eight forms recognized in the qualitative characters and the CVAs were compared in each floral quantitative character or ratio of several characters to examine their discreteness among the forms, and to find diagnostic characters between them.

1. Size and shape of calyx tube (CTL, CTW, CTTW, and OP) In CTL and CTW, which represent the size of calyx tube, the ranges of form D4 were conspicuously lower than those of the other forms, and were almost discontinuous especially with the other D forms (Figs. 8A, 8B). In CTL form D3 was conspicuously higher than the other D forms, and that of L4 was lower than the other L forms, especially almost discontinuous with form L2 (Fig. 8A). In CTW, form L4 was larger than the other L forms (Fig. 8B). In ratio CTL/CTW (Fig. 8C), the

range of form D3 was almost discontinuously higher than those of the other D forms, and that of form L4 was almost discontinuously lower than the other L forms. Those of L forms except form L4 were generally higher than those of D forms except form D3.

In the range of CTTW, the eight forms were divided into two groups; those of form, D1, D2, D4, L4, and L2 were larger, and those of forms D3, L1, and L3 were smaller (Fig. 8D). The range of ratio CTTW/CTW divided the two groups more effectively, nearly discontinuously with value 0.55 as boundary (Fig. 8E).

In OP, which represents ovule position, the range of form L3 was lower than those of the other forms, and was almost discontinuous with those of all D forms and form L4 (Fig. 8F).

2. Size and shape of calyx lobe (CLD, CLL, CLW, CLFW, CLTL, and CLTD) In CLD (Fig. 8G), which represents the condition of calyx lobes, e.g. recurved, patent, or erect, the range of D forms were wider than those of L forms and highly overlapped each other within D forms. On the other hand, L forms were divided into two groups; those of form L4 and L1 were lower, and those of form L2 and L3 were higher. It should be noted that the range of form L1 was broader than those of the other L forms, from highly recurved to patent. These results indicate that the condition of calyx lobes is not always stable though Maekawa (1936b), Ohwi (1956), and Satake and Momiyama (1982) applied it to discriminate *A. heterotropoides* from *A. sieboldii*.

CLL, CLW, and CLFW represent the size of calyx lobes. In CLL, the range of form L4 and L1 were lower than those of the other forms, almost discontinuous with form L3 (Fig. 8H). In CLW, form L1 had lower range than the other L forms (Fig. 8I). In CLFW, the range of form D3, L1, and L3 were lower than those of the other forms (Fig. 8J); such trends were similar to those of CTTW/CTW (Fig. 8E). In ratio CLL/CLW, which represents the aspect ratio of calyx lobes, the range of form L4 was almost distinctly lower, and that of form D3 was almost discontinuously higher than those of the other forms (Fig. 8K). The range of CLFW/CLW, which represents the degree from pentangular to deltoid in outline of calyx lobe, showed that form L4 had deltoid calyx lobes though the other forms had pentangular ones, and that form L3 was almost distinct from the other L forms in calyx lobe shape (Fig. 8L).

Almost all individuals of form L4, and many individuals of form L1 had no or little part corresponding to CLTL, which represents the degree of tapering (obtuse to acuminate) in calyx lobes at apex (Fig. 8M). On the other hand, almost all individuals in the other forms had this part. Although apical shape of calyx lobes is one of the discriminant characters between *A. sieboldii* and *A. heterotropoides* var. *heterotropoides* (Schmidt 1868, Maekawa 1936b, Ohwi 1956, Satake and Momiyama 1982), considerable numbers of samples in Hokkaido belonging to *A. heterotropoides* var. *heterotropoides* had acuminate calyx lobes with acuminate apices (Yamaji et al. 2006).

In CLTD, the range of form D3 was lower but overlapped than those of the other forms, and that of form L4 was almost discontinuously higher than those of the other forms (Fig. 8N).

3. Pistil characters (PPL and PPI) PPL (Fig. 8O) was adopted for one of the discriminant characters for sect. Asiasarum plants in Korea (Yamaki et al. 1996). The ranges of PPL of L forms, excluding form L4 were lower than those of D forms exclude form D3. The range of form L4 was higher than those of the other L forms, and was almost discontinuous with that of form L2. The ranges of PPI were, however, highly overlapped among the all forms (Fig. 8P).

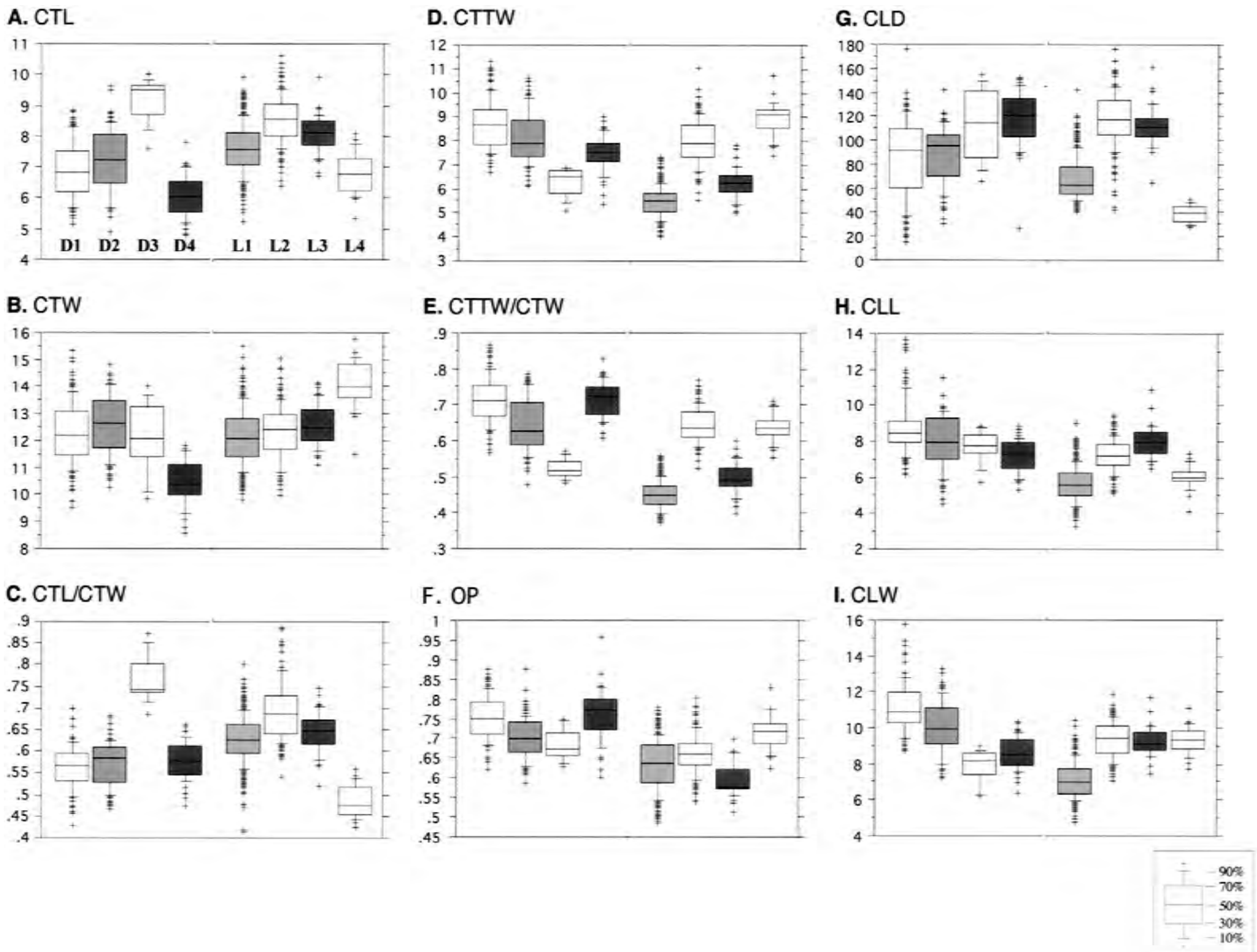

Fig. 8. Box and whisker plot for each quantitative character or proportions in the eight forms of *Asarum* sect. Asiasarum recognized in this study. From the left to right, form D1, D2, D3, D4, L1, L2, L3, and L4.

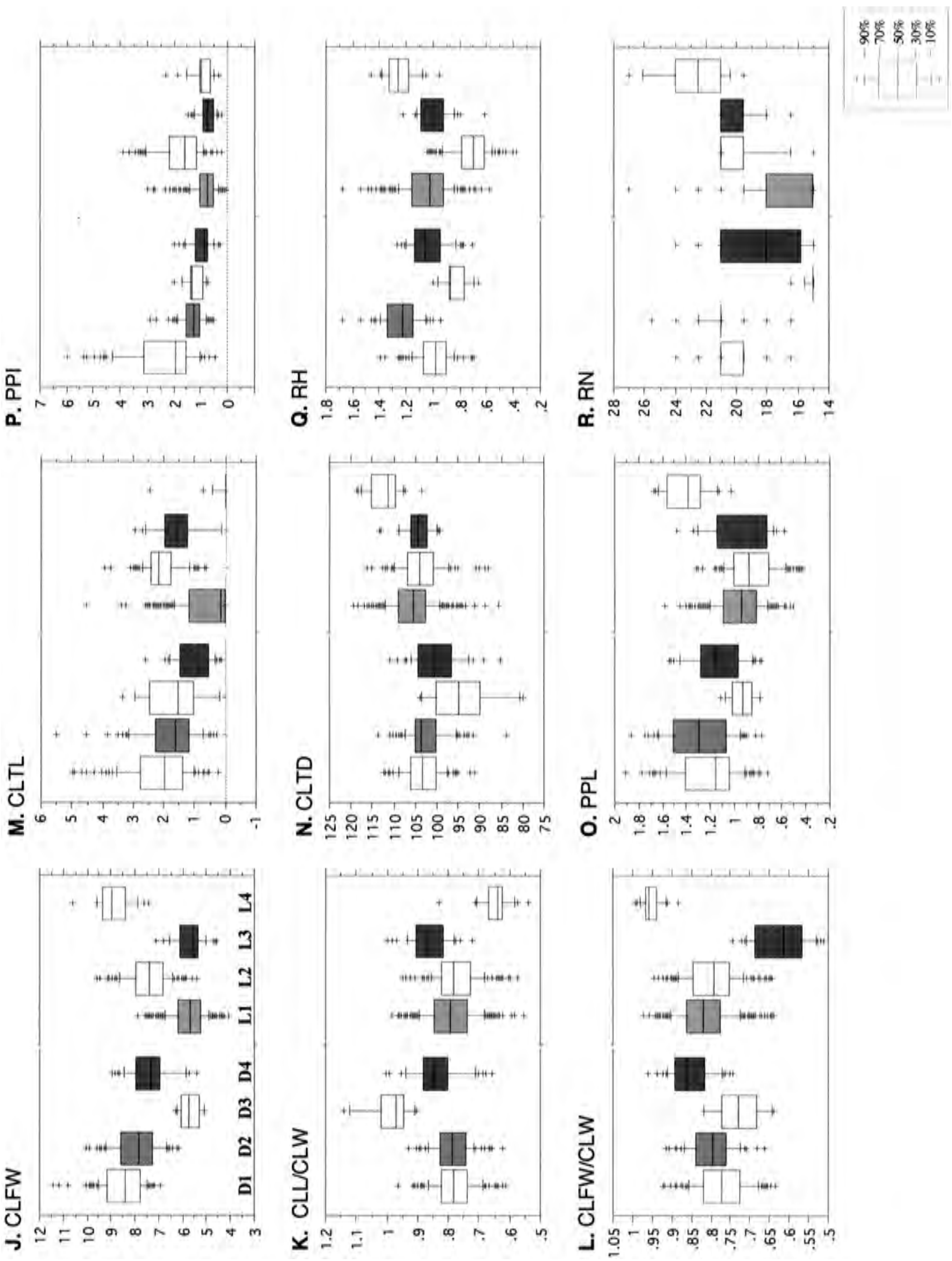

Fig. 8. Continued.

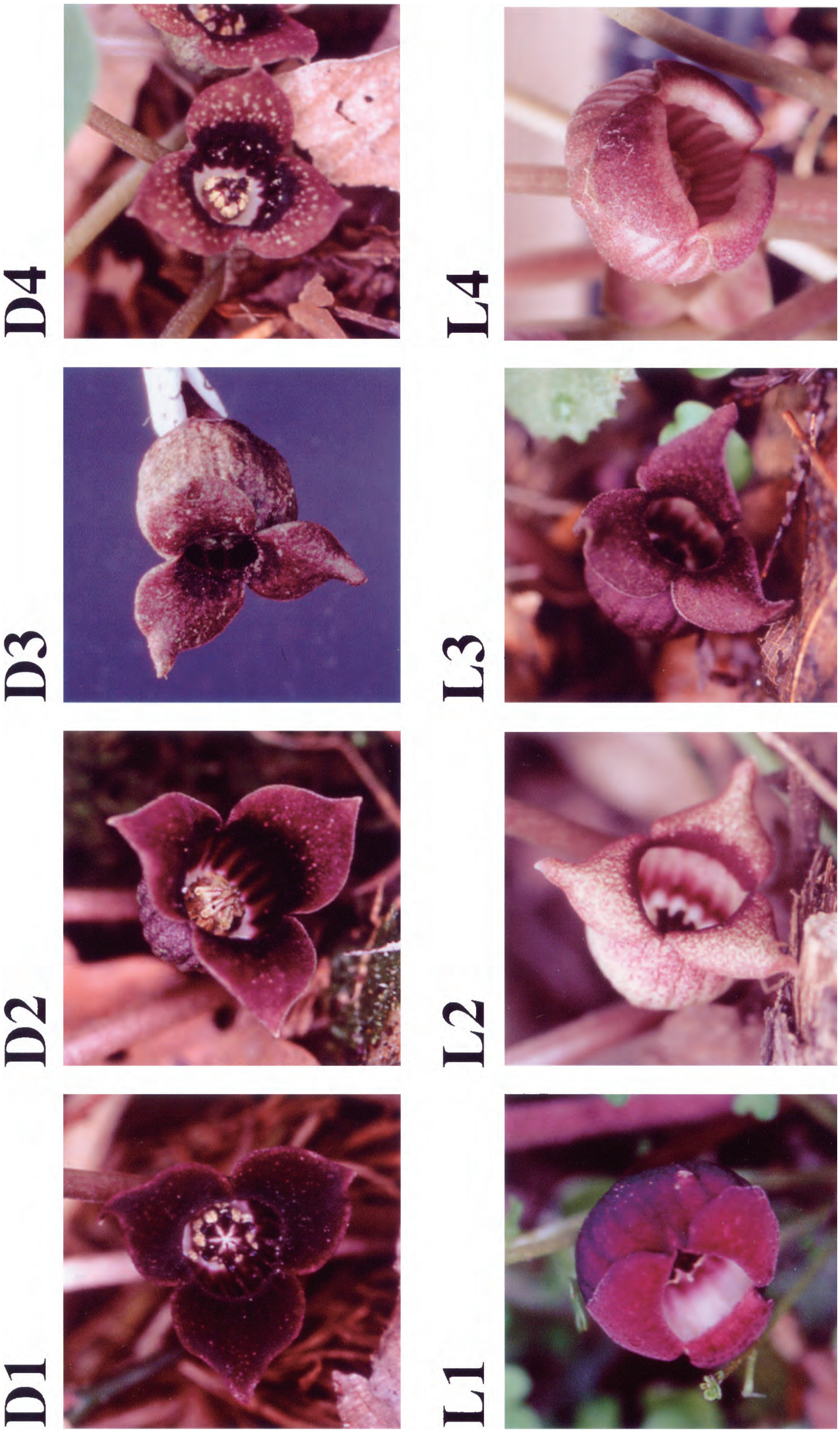

Fig. 9. Comparison between fresh flowers for the eight forms of *Asarum* sect. Asiasarum plants recognized in this study. Form D1 (No. 41), D2 (No. 50), D3 (No. 49), D4 (No. 51), L1 (No. 15), L2 (No. 35). L3 (No. 33), and L4 (No. 54).

4. Ridges in inner surface of calyx tube (RH and RN) In RH, the ranges of the eight forms were different from each other (Fig. 8Q). It is noteworthy that those of form D1 and D2 were markedly different, though there were little conspicuous differences in the other quantitative characters. In particular, that of form L2 was almost discontinuously lower than those of the other L forms. In RN (Fig. 8R), the range of form D3 was lowest among those of all the eight forms and almost discontinuous with those of the other forms except forms D4 and L1, and that of form L4 was higher than those of the other forms and almost discontinuous with those of forms D3 and L1.

Discussion

In this study, we recognized the eight morphologically distinct forms belonging to *Asarum* sect. Asiasarum in Japan (Fig. 9). Their distributions were almost allopatric (Fig. 6). Form D1 was recognized in central Honshu; form D2 was from the west of central Honshu, form D3 was in a restricted area of western Honshu, and form D4 was in Shikoku and Kyushu. Form L1 was recognized in Hokkaido, northern Honshu, form L2 was in north of central Honshu, form L3 was in restricted area of central Honshu, and form L4 was in restricted area of central Kyushu. Although a few intermediate individuals were exceptionally recognized in the CVAs, we conclude that the all eight forms are worthy to put distinct taxa.

Fourteen of 55 populations used for the detailed observation of floral characters were also examined in the previous studies (Nakamura et al. 1979, 1987, Nakamura 1986, Nakamura and Nagasawa 1987, Table 2), and those populations covered all taxa recognized by Nakamura (1986). As the result of comparison in these populations, each form corresponded to only the single form in Nakamura (1986): forms D1 and D2 = "Western Honshu type" *A. sieboldii*, D3 = "San'in type" *A. sieboldii*, D4 = *A. dimidiatum*, L1 = *A. heterotropoides* var. *heterotropoides*, L2 = "Tohoku type" *A. sieboldii*, L3 = "Tanigawa type" *A. sieboldii*, and L4 = "Aso type" *A. sieboldii*. There is no discrepancy except that "Western Honshu type" *A. sieboldii* separated into form D1 and D2. Nakamura (1986) noted that "Western Honshu type" *A. sieboldii* with multicellular trichomes of flower, which is the diagnostic character of form D2, were exceptional because they were recognized in only a few populations, however, form D2 is commonly distributed in the west of central Honshu.

This study investigated the effectiveness of detailed measurements of quantitative characters and multivariate analyses for classification of plants. Like sect. Asiasarum, in case of a group of plants which is difficult to classify by single or a few quantitative characters, e. g., size, shape, and ratio of two characters, multivariate analysis is able to discriminate more than one forms by their differences in their general shape. In this study, one of the multivariate analyses, canonical variates analysis (CVA; Wiley 1991) proved effective at discriminating of four forms in forms L.

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References

Cheng C. Y. and Yang C. S. 1983. A synopsis of the

Chinese species of *Asarum* (Aristolochiaceae). J. Arn. Arb. **64**: 565–597.
Hatusima S. 1993. *Asarum heterotropoides* var. *mandshuricum* newly found in Japan. J. Phytogeogr. Taxon **41**: 69–70.
Kelly L. M. 1997. A cladistic analysis of *Asarum* (Aristolochiaceae) and implications for the evolution of herkogamy. Amer. J. Bot. **84**: 1752–1765.
Lee Y. N. and Lee J. Y. 2000. *Asarum* in Korea. Bull. Korea Pl. Res. **1**: 16–30.
Maekawa F. 1936a. Japanese Asaraceae (IX). J. Jpn. Bot. **12**: 28–35.
—— 1936b. Aristolochiaceae. *In*: Nakai T. (ed.), Flora Sylvatica Koreana **21**: 1–28.
—— 1956. Phylogeny of plants. Shizen **11**: 12–20 (in Japanese).
Nagasawa M. 1961. Analysis of crude drugs by infrared spectrophotometry II. Analysis of radix Asiasari by infrared spectrophotometry. Yakugaku Zasshi **81**: 129–138 (in Japanese).
Nakamura T. 1979. Geographic variation of *Asiasarum sieboldii* (Miq.) F. Maek. Shuseibutsugaku-kenkyu **3**: 70–77 (in Japanese).
—— 1986. Taxonomical studies of Japanese *Asiasarum* (Aristolochiaceae) with special reference to geographical variation in the morphology and chemical composition. Dr. thesis. Tokyo Metropolitan University.
—— and Nagasawa M. 1987. Variation in pollen grains of Japanese *Asiasarum* (Aristolochiaceae). J. Jpn. Bot. **62**: 134–138.
——, Endo J. and Hamada T. 1982. Variation of *Asiasarum dimidiatum*. J. Jpn. Bot. **57**: 366–375 (in Japanese).
——, —— and Nagasawa M. 1979. Geographical variation of the essential oil components in *Asiasarum heterotropoides*. J. Jpn. Bot. **54**: 334–341.
——, —— and —— 1987. Geographical variation in essential oil composition of *Asiasarum sieboldii* (Aristolochioaceae). J. Jpn. Bot. **62**: 7–16.
Oh B., Nam O. and Kim J. 1997. A new species of *Asarum* sect. *Asiasarum* from Korea: *A. misandrum* B. Oh et J. Kim. Kor. J. Plant Tax. **27**: 491–499.
Ohwi J. 1965. Flora of Japan (in English ; Meyer, F. G. and Walker, E. H. (eds.)). Smithsonian Institution, Washington, D. C.
Satake Y. and Momiyama S. 1982. Aristolochiaceae. *In*: Satake Y. et al. (eds.), Wild Flowers of Japan, Herbaceous plants. **II**: 102–109. Heibonsha, Tokyo (in Japanese).
Schmidt F. 1868. Reisen im Amur-Lande und auf der Insel Sachalin, p.171. Mémoires de l'Académie Impériale des Scienses de St.-Pétersbourg, VII Serie. Tome XII, No. 2.
Wiley E. O. 1991. Phylogenetics–The theory and practice of phylogenetic systematics (Japanese edition: Miya M., Nishida S. and Okiyama M. translated). Tokyo: Bunichi-sogoshuppan.
Yamaji H., Yokoyama J., Ohashi H. and Maki M. 2006. Concordant clines and significant correlation between floral and pollen characters in *Asarum heterotropoides* var. *heterotropoides* (Aristolochiaceae). Pl. Syst. Evol. **259**: 1–17.
Yamaki K., Terabayashi S., Okada M. and Pak J.-H. 1996. A new species and new variety of *Asiasarum* (Aristolochiaceae) from Korea. J. Jpn. Bot. **71**: 1–10.

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花部形態の多変量解析に基づく日本産カンアオイ属ウスバサイシン節の形態比較

日本産カンアオイ属ウスバサイシン節植物の形態変異を明らかにするために，国内の全分布域・既知の分類群，地域集団を含む55集団について野外調査を行った．同節に関する過去の研究はいずれも量的形質の評価が不十分であるため，本研究では今まで用いられてきた形質，新たに採用した形質の評価に加え，花の量的形質に基づく多変量解析を行った．その結果，形態より区別できる8型が認識された．日本の同節はまず萼筒内壁のカラーパターンで2型に分けられ，D型は全面暗紫色なのに対し，L型は基底部は暗紫色，中央部は黄緑色ないし淡紫色，萼筒開口部は暗紫色ないし白色だった．D型はさらに萼筒内壁，萼裂片内面の毛の細胞数，雄蕊・雌蕊の数でD1–D4の4型に分けられ，L型は萼筒の形態，萼筒開口部の大きさ，萼裂片の形態，サイズでL1–L4の4型に分けられた．この8型はほぼ異所的に分布し，それぞれ独立の分類群に値するまとまった地域集団と推定された．

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